

## **Acute sensitivity of Ph-like acute lymphoblastic leukemia to the SMAC-mimetic birinapant**

Jennifer Richmond<sup>1</sup>, Alissa Robbins<sup>1</sup>, Kathryn Evans<sup>1</sup>, Dominik Beck<sup>2</sup>, Raushan T. Kurmasheva<sup>3</sup>, Catherine A. Billups<sup>4</sup>, Hernan Carol<sup>1</sup>, Sue Heatley<sup>5</sup>, Rosemary Sutton<sup>1</sup>, Glenn M. Marshall<sup>6</sup>, Deborah White<sup>5</sup>, John Pimanda<sup>2</sup>, Peter J. Houghton<sup>3</sup>, Malcolm A. Smith<sup>7</sup>, and Richard B. Lock<sup>1</sup>

<sup>1</sup>Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Australia, Sydney, Australia

<sup>2</sup>Lowy Cancer Research Centre, UNSW Australia, Sydney, Australia

<sup>3</sup>Greehey Children's Cancer Research Institute, University of Texas Health Science Center San Antonio, San Antonio, Texas

<sup>4</sup>Department of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee

<sup>5</sup>South Australia Health and Medical Research Institute, Adelaide, South Australia, Australia

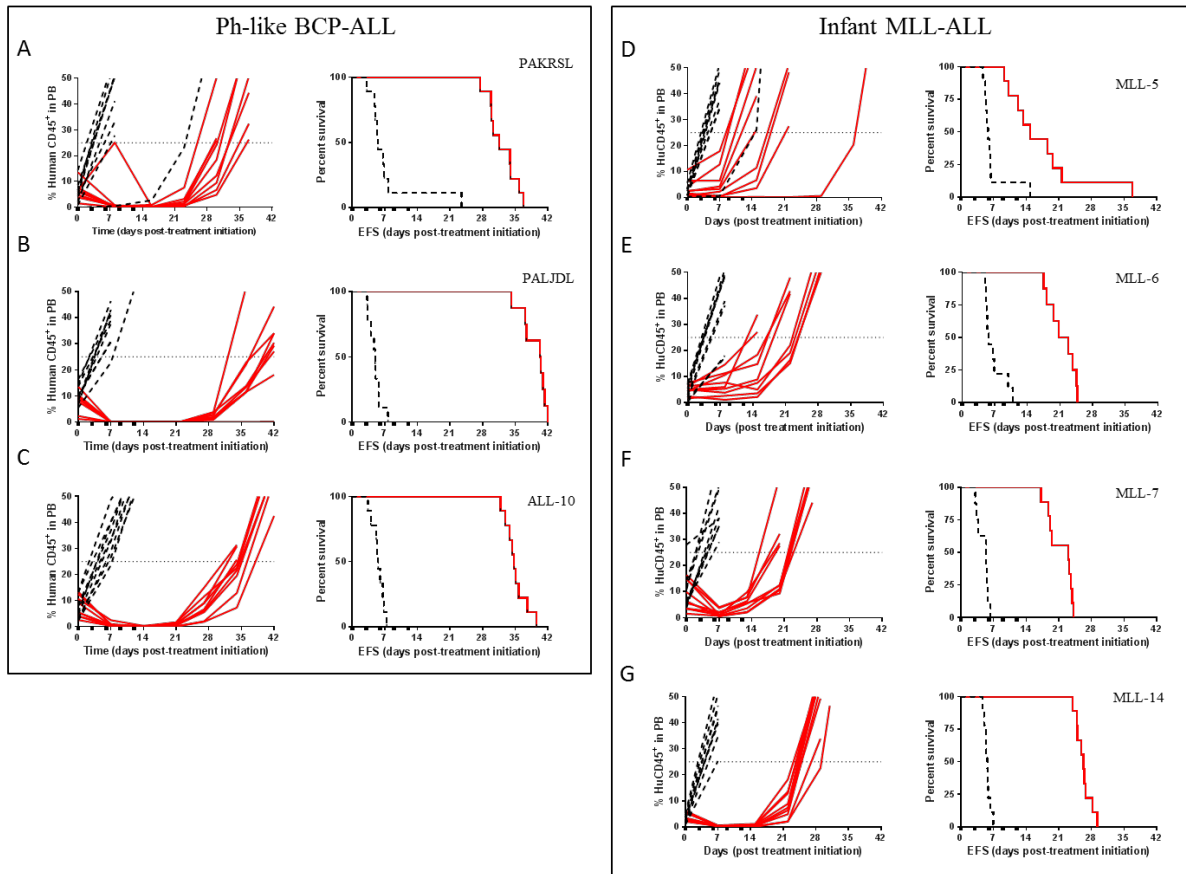
<sup>6</sup>Kids Cancer Centre, Sydney Children's Hospital, Randwick, Australia

<sup>7</sup>Cancer Therapy Evaluation Program, NCI, Bethesda, Maryland

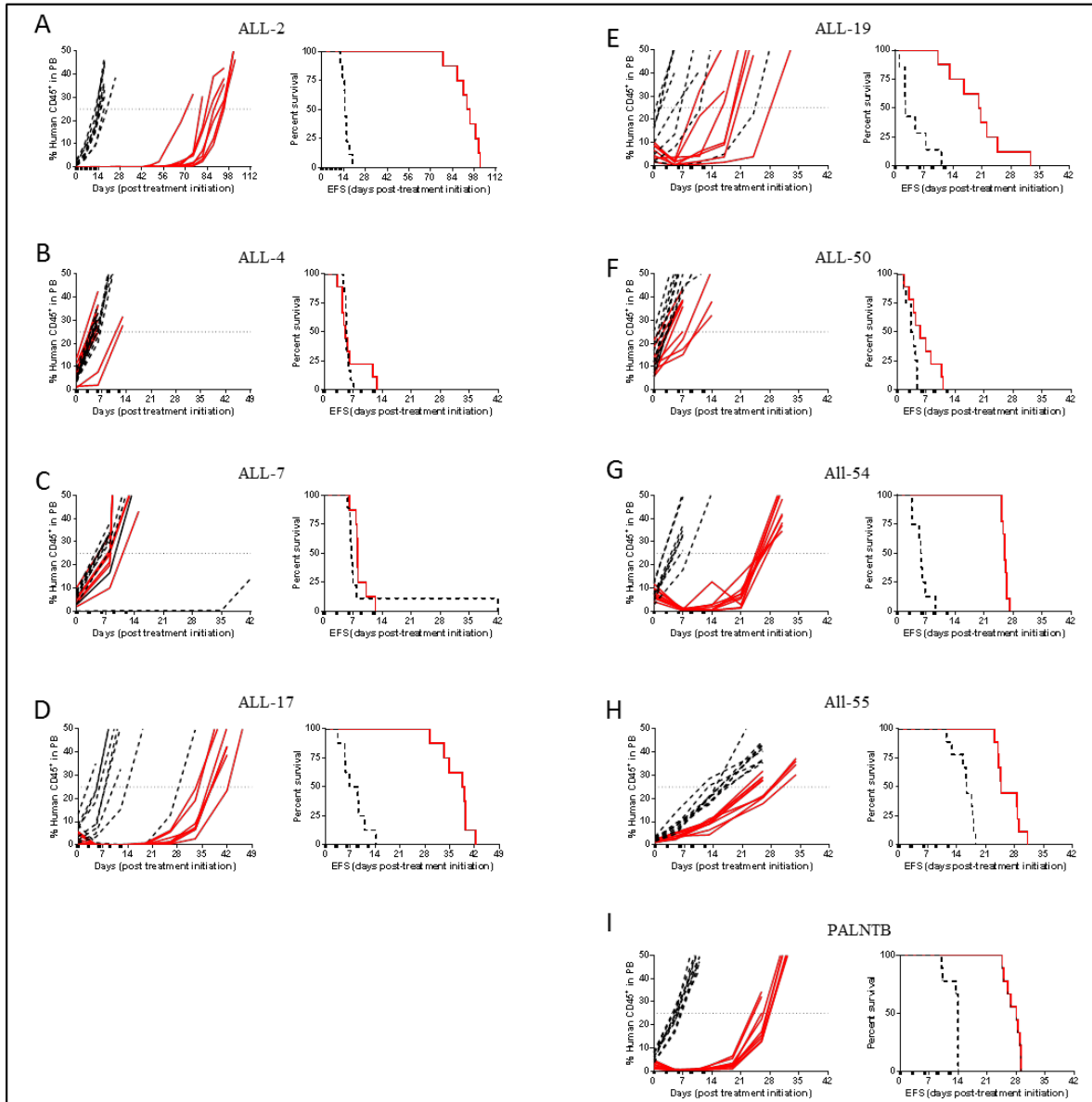
## **SUPPLEMENTAL FIGURES AND TABLES**

# Supplemental Figures

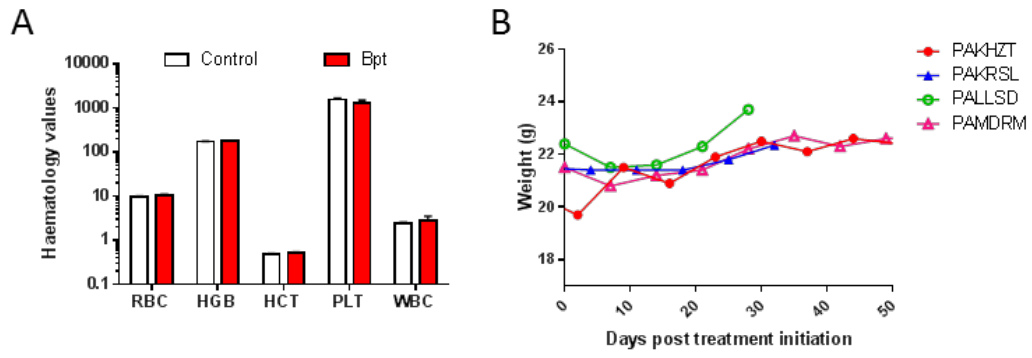
## Supplemental Figure S1



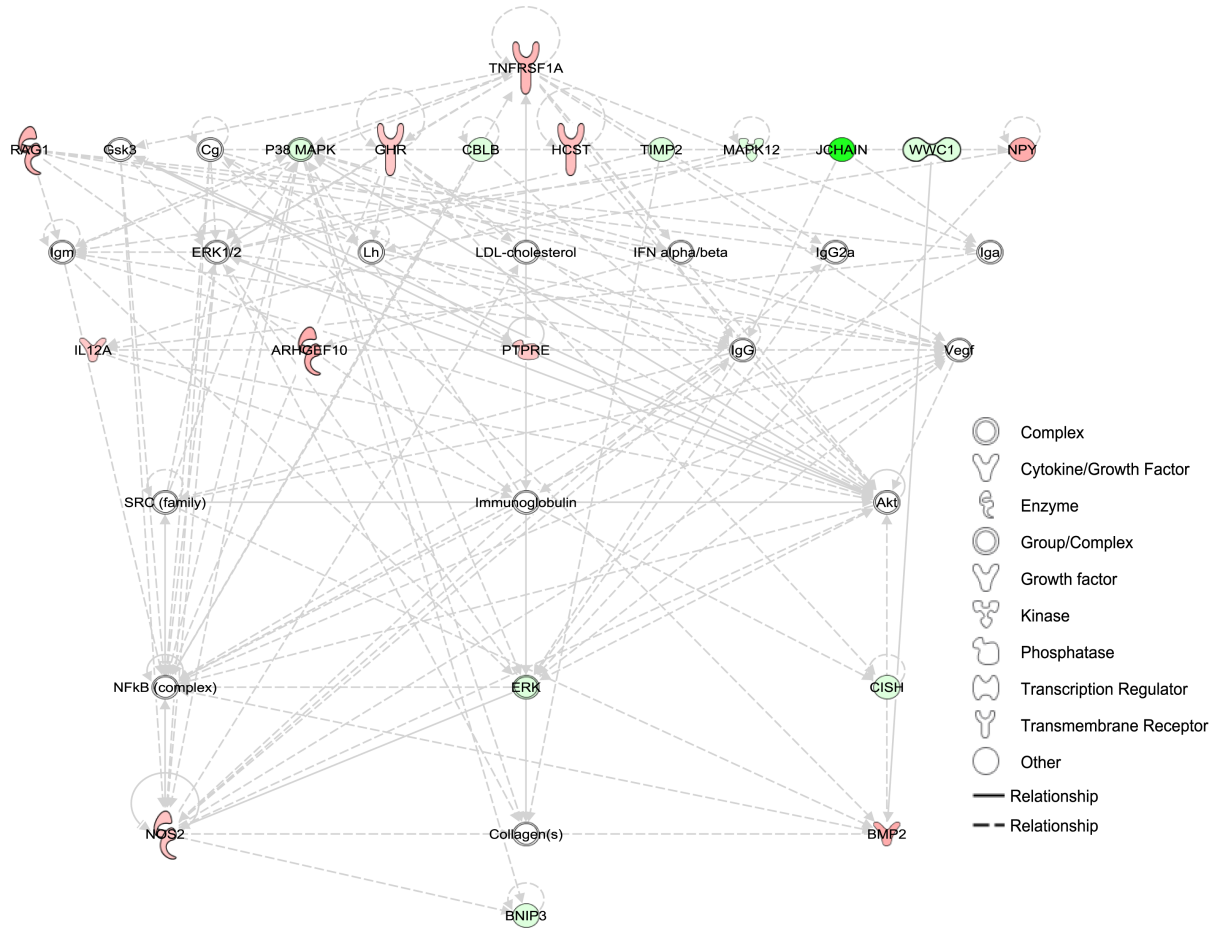
# Supplemental Figure S2



# Supplemental Figure S3



# Supplemental Figure S4



## Supplementary Tables

**Table S1. Patient demographics of primary patient samples and PDXs**

Patient sample/PDX	Age (y)	Sex	Lineage	Disease Status at Biopsy	Cytogenetics	Specific Kinase Lesions	CRLF2 Expression <sup>1</sup>	CRLF2 Rearrangement <sup>2</sup>	Ph-like <sup>3</sup>
#102d	3.1	M	BCP-ALL	Diagnosis	47, XY, +X[14]/46, XY[9]	JAK2 T875N	High	<i>P2RY8-CRLF2</i>	Y
#102r	5.3	M	BCP-ALL	Relapse	Not available	JAK2 T875N	High	<i>P2RY8-CRLF2</i>	Y
#103	16.7	F	BCP-ALL	Diagnosis	50,XX,+X,+13,+17,+22[4]/46,XX[11]	Not detected	High	<i>P2RY8-CRLF2</i>	Y
#106	14.9	M	BCP-ALL	Diagnosis	46,XY	<i>EBF1-PDGFRB</i>	Normal	None	Y
#107	5.5	M	BCP-ALL	Diagnosis	47,XY,+X[14]/46,XY[6]	JAK2 R683S	High	<i>P2RY8-CRLF2</i>	Y
#108	6.2	F	BCP-ALL	Diagnosis	46,XX,dic(9;20)(p13;q11.2),del(11)(q13q22),+21[12]/46,XX[8]	Not detected	High	<i>P2RY8-CRLF2</i>	Y
#109	66	M	BCP-ALL	Diagnosis	Trisomy Chr 5&13	FLT3 V579A	High	<i>IGH@-CRLF2</i>	Y
#110	37	F	BCP-ALL	Diagnosis	t(9;22)	<i>BCR-ABL1</i>	Normal	None	N
#111	28	M	BCP-ALL	Diagnosis	add(9)(p24)	<i>ATF7IP-JAK2</i>	Normal	None	Y
#112	18	M	BCP-ALL	Diagnosis	Not available	Not detected	Normal	None	N
#113	16	M	BCP-ALL	Diagnosis	Normal	Not detected	Normal	None	N
#114	81	M	BCP-ALL	Diagnosis	TEL-ABL	<i>ETV6-ABL1</i>	Normal	None	Y
ALL-10	4	M	BCP-ALL	Diagnosis	47,XY,+mar	JAK1 V658L	High	Not done	Y
PAKHZT	13.9	M	BCP-ALL	Diagnosis	Not available	JAK2 R867Q	High	<i>IGH@-CRLF2</i>	Y
PAKRSL	13.7	F	BCP-ALL	Diagnosis	Not available	JAK2 R683G	High	<i>IGH@-CRLF2</i>	Y
PAKSWW	15.1	M	BCP-ALL	Diagnosis	Not available	JAK1 V658F	High	<i>IGH@-CRLF2</i>	Y
PALJCF	3.5	F	BCP-ALL	Diagnosis	Not available	JAK1 L624_R629>W	High	<i>P2RY8-CRLF2</i>	Y
PALNTB	8.7	F	BCP-ALL	Diagnosis	Not available	JAK2 P933R	High	<i>IGH@-CRLF2</i>	N
PAMDRM	7.9	M	BCP-ALL	Diagnosis	Not available	JAK2 GPins1682	High	<i>IGH@-CRLF2</i>	Y
PALJDL	3.2	M	BCP-ALL	Diagnosis	Not available	IL7R p.L242_L243insFPGVC mutation; SH2B3 e1-2 deletion	Normal	Not done	Y
PALLSD	14.2	M	BCP-ALL	Diagnosis	Not available	JAK2 R683G	High	<i>IGH@-CRLF2</i>	Y
PAKVKK	14.4	M	BCP-ALL	Diagnosis	Not available	<i>NUP214-ABL1</i>	Normal	Not done	Y
PALTWS	<18	M	BCP-ALL	Diagnosis	Not available	Not detected	High	<i>IGH@-CRLF2</i>	N
ALL-2	5.5	F	BCP-ALL	Relapse	Normal	FLT3 Y572S	Normal	Not done	N
ALL-4	8.9	M	BCP-ALL	Diagnosis	t(9;22)	<i>BCR-ABL1</i>	Normal	Not done	N
ALL-7	7.4	M	BCP-ALL	Diagnosis	t(17;19), <i>TCF3-HLF</i>	Not detected	Normal	Not done	N
ALL-11	3.1	F	BCP-ALL	Diagnosis	del(12)(p13) del(13)(q12)	Not detected	Not done	Not done	N
ALL-17	8.11	F	BCP-ALL	Diagnosis	Normal	Not detected	Normal	Not done	N
ALL-19	16.2	M	BCP-ALL	Relapse	Normal	<i>NUP214-ABL1</i>	Normal	Not done	N
ALL-50	10.8	M	BCP-ALL	Diagnosis	Normal	Not detected	Normal	Not done	N
ALL-54	7.3	M	BCP-ALL	Diagnosis	Normal	Not detected	Not done	Not done	N
ALL-55	14.5	M	BCP-ALL	Diagnosis	t(9;22)(q34;q11.2)	<i>BCR-ABL1</i>	Normal	Not done	N
ALL-56	10	M	BCP-ALL	Diagnosis	t(9;22)(q34;q11.2)	<i>BCR-ABL1</i>	Not done	Not done	N

ALL-57	5.9	F	BCP-ALL	Diagnosis	t(1;19)(q23;p13), <i>TCF3-PBX1</i>	Not detected	Normal	Not done	N
MLL-2	< 1	M	BCP-ALL	Unknown	t(4;11), <i>MLL-AFF1</i>	Not detected	Normal	Not done	Not done
MLL-3	< 1	M	BCP-ALL	Unknown	t(11;17), <i>MLL-GAS7</i>	Not detected	Normal	Not done	Not done
MLL-5	< 1	M	BCP-ALL	Unknown	t(10;11), <i>MLL-MLLT10</i>	Not detected	Moderate	Not done	Not done
MLL-6	< 1	M	BCP-ALL	Unknown	t(11;19), <i>MLL-MLLT1</i>	Not detected	Normal	Not done	Not done
MLL-7	< 1	M	BCP-ALL	Diagnosis	t(4;11), <i>MLL-AFF1</i>	Not detected	Normal	Not done	Not done
MLL-8	< 1	F	BCP-ALL	Unknown	t(11;19), <i>MLL-MLLT1</i>	Not detected	Normal	Not done	Not done
MLL-14	< 1	F	BCP-ALL	Unknown	t(11;19), <i>MLL-MLLT1</i>	Not detected	Normal	Not done	Not done
ALL-8	12.8	M	T-ALL	Relapse	Normal	Not detected	Not done	Not done	Not done
ALL-16	10.2	F	T-ALL	Diagnosis	Normal	Not detected	Not done	Not done	Not done
ALL-27	8.5	M	T-ALL	Diagnosis	Normal	Not detected	Normal	Not done	Not done
ALL-29	4.9	M	T-ALL	Diagnosis	46,XY	Not detected	Normal	Not done	Not done
ALL-30	7.4	M	T-ALL	Diagnosis	Not available	Not detected	Normal	Not done	Not done
ALL-31	10.2	M	T-ALL	Diagnosis	del(6)(q21),del(11)(q23)	Not detected	Normal	Not done	Not done
ALL-32	11.1	M	T-ALL	Relapse	del(9)(q34)	Not detected	Normal	Not done	Not done
ALL-42	2.6	M	T-ALL	Diagnosis	Normal	Not detected	Normal	Not done	Not done
ALL-43	6.2	M	T-ALL	Diagnosis	Normal	Not detected	Normal	Not done	Not done
ETP-12	16	M	ETP-ALL	Diagnosis	Not available	<i>SH2B3</i> SV	Not done	Not done	Not done
ETP-13	3	M	ETP-ALL	Diagnosis	Not available	Not detected	Not done	Not done	Not done
ETP-14	14	M	ETP-ALL	Diagnosis	Not available	JAK1 S703I; PTEN deletion	Not done	Not done	Not done

<sup>1</sup> by mRNA expression; <sup>2</sup> by PCR; <sup>3</sup> as define in Harvey RC, et al. *Blood*. 2010;116(23):4874-84; Mullighan CG, et al. *Proc Natl Acad Sci USA*. 2009;106(23):9414-8; Roberts KG, et al. *N Engl J Med*. 2014;371(11):1005-15; Roberts KG, et al. *Cancer Cell*. 2012;22(2):153-66; Yeung DT, et al. *Leukemia*. 2015;29(1):230-2.

**Table S2. Single agent efficacy of birinapant against pediatric ALL PDX models *in vivo*.**

Xenograft	ALL subtype	Dose (mg/kg)	Median EFS		T-C (days)	Log-rank P-value	Overall Response	
			Control	Bpt				
<b>ALL-2</b>	BCP-ALL	30	14.8	91.7	76.9	<0.001	<b>MCR</b>	
<b>ALL-4</b>	BCP-ALL	30	5.3	5.1	-0.2	0.816	<b>PD1</b>	
<b>ALL-7</b>	BCP-ALL	30	6.6	8.6	2	0.238	<b>PD1</b>	
<b>ALL-17</b>	BCP-ALL	30	7.9	39	31.1	<0.001	<b>CR</b>	
<b>ALL-19</b>	BCP-ALL	30	5	20.6	15.6	0.001	<b>SD</b>	
<b>ALL-50</b>	BCP-ALL	30	3.6	5.6	2	0.026	<b>PD2</b>	
<b>ALL-54</b>	BCP-ALL	30	5.7	26	20.3	<0.001	<b>PR</b>	
<b>ALL-55</b>	BCP-ALL	30	16.5	25.4	8.9	<0.001	<b>PD2</b>	
<b>PALNTB</b>	BCP-ALL	30	5.7	28	22.3	<0.001	<b>CR</b>	
<b>ALL-10</b>	Ph-like BCP-ALL	30	5.6	34.8	29.2	<0.001	<b>CR</b>	
<b>PAKHZT</b>	Ph-like BCP-ALL	30	12.8	60.2	47.4	<0.001	<b>MCR</b>	
<b>PAKRSL</b>	Ph-like BCP-ALL	30	5.5	31.6	26.1	<0.001	<b>CR</b>	
<b>PALJDL</b>	Ph-like BCP-ALL	30	4.9	40.5	35.6	<0.001	<b>CR</b>	
<b>PALLSD</b>	Ph-like BCP-ALL	30	4.9	29.8	24.9	<0.001	<b>CR</b>	
<b>PAMDRM</b>	Ph-like BCP-ALL	30	8.5	73.2	64.7	<0.001	<b>MCR</b>	
<b>MLL-5</b>	Infant MLL-ALL	30	5.8	14.8	9	0.001	<b>PD2</b>	
<b>MLL-6</b>	Infant MLL-ALL	30	5.8	21.2	15.4	<0.001	<b>PD2</b>	
<b>MLL-7</b>	Infant MLL-ALL	30	5.4	23.1	17.7	<0.001	<b>PD2</b>	
<b>MLL-14</b>	Infant MLL-ALL	30	5.7	26.3	20.6	<0.001	<b>CR</b>	
			3.8	4.9	10.4	5.5	<0.001	<b>PD2</b>
<b>PALLSD</b>	Ph-like BCP-ALL	7.5	4.9	17.6	12.7	<0.001	<b>PD2</b>	
		15	4.9	21.9	17	<0.001	<b>PR</b>	
		3.8	8.5	33.1	24.6	<0.001	<b>CR</b>	
<b>PAMDRM</b>	Ph-like BCP-ALL	7.5	8.5	50.9	42.4	<0.001	<b>CR</b>	
		15	8.5	65.2	56.7	<0.001	<b>MCR</b>	



**Table S3. Individual mouse responses to birinapant as per PPTP format**

Xenograft	Animal Counts/Statistics						EFS Evaluation			Response Evaluation							Overall Response	
	Group	N1 <sup>3</sup>	Nd <sup>4</sup>	No <sup>5</sup>	Na <sup>6</sup>	No.ev <sup>7</sup>	KM med. <sup>8</sup>	Log-Rank P-value	EFS T/C	PD	PD1	PD2	SD	PR	CR	MCR	Median Score	
ALL-2	A <sup>1</sup>	9	0	0	9	9	14.8			9	0	0	0	0	0	0	0	PD
	B <sup>2</sup>	8	0	0	8	8	91.7	<0.001	6.2	0	0	0	0	0	0	8	10	MCR
ALL-4	A	10	0	0	10	10	5.3			10	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	5.1	0.816	1	0	7	2	0	0	0	0	0	PD1
ALL-7	A	9	0	0	9	8	6.6			8	0	0	1	0	0	0	0	PD
	B	9	1	0	8	8	8.6	0.238	1.3	0	6	2	0	0	0	0	0	PD1
ALL-17	A	8	0	0	8	8	7.9			8	0	0	0	0	0	0	0	PD
	B	8	0	0	8	8	39	<0.001	4.9	0	0	0	0	0	8	0	8	CR
ALL-19	A	8	0	1	7	7	5			7	0	0	0	0	0	0	0	PD
	B	8	0	1	7	7	20.6	0.001	4.1	0	0	4	0	3	0	0	2	PD2
ALL-50	A	9	0	0	9	9	3.3			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	5.6	0.026	1.7	0	4	5	0	0	0	0	2	PD2
ALL-54	A	8	0	0	8	8	5.7			8	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	26	<0.001	4.5	0	0	0	0	6	3	0	6	PR
ALL-55	A	9	0	0	9	9	16.5			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	25.4	<0.001	1.5	0	1	8	0	0	0	0	2	PD2
PALNTB	A	9	0	0	9	9	5.7			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	28	<0.001	4.9	0	0	0	0	1	8	0	8	CR
PAKHZT	A	9	0	0	9	9	12.8			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	60.2	<0.001	4.7	0	0	0	0	0	3	6	10	MCR
PAKRSL	A	9	0	0	9	9	5.5			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	31.6	<0.001	5.7	0	1	0	0	1	7	0	8	CR
PAMDRM	A	7	0	0	7	7	8.5			7	0	0	0	0	0	0	0	PD
	B	7	0	1	6	6	73.2	<0.001	8.6	0	0	0	0	0	0	6	10	MCR
PALJDL	A	9	0	0	9	9	4.9			9	0	0	0	0	0	0	0	PD
	B	9	0	1	9	8	40.5	<0.001	8.2	0	0	0	0	0	8	0	8	CR
ALL-10	A	9	0	0	9	9	5.6			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	34.8	<0.001	6.2	0	0	0	0	0	9	0	8	CR
PALLSD	A	8	0	0	8	8	4.9			8	0	0	0	0	0	0	0	PD
	B	8	0	0	8	8	29.8	<0.001	6	0	0	0	0	0	8	0	8	CR
MLL-5	A	9	0	0	9	9	5.8			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	14.8	0.001	2.5	0	0	6	0	2	1	0	2	PD2
MLL-6	A	9	0	0	9	9	5.8			9	0	0	0	0	0	0	0	PD
	B	9	0	1	8	8	21.2	<0.001	3.7	0	0	8	0	0	0	0	2	PD2
MLL-7	A	8	0	0	8	8	5.4			8	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	23.1	<0.001	4.2	0	0	6	0	3	0	0	2	PD2
MLL-14	A	9	0	0	9	9	5.7			9	0	0	0	0	0	0	0	PD
	B	9	0	0	9	9	26.3	<0.001	4.6	0	0	0	0	2	7	0	8	CR
PAMDRM	3.8 mg/kg	8	0	0	8	8	33.1	0.00062	3.9	0	0	0	0	1	7	0	8	CR
	7.5 mg/kg	7	0	0	7	7	50.9	0.00058	6	0	0	0	0	0	7	0	8	CR
	15 mg/kg	8	0	0	8	8	65.2	0.00062	7.6	0	0	0	0	0	0	8	10	MCR
PALLSD	3.8 mg/kg	8	0	0	8	8	10.4	0.00016	2.1	0	0	8	0	0	0	0	2	PD2
	7.5 mg/kg	8	0	0	8	8	17.6	0.00016	3.6	0	0	8	0	0	0	0	2	PD2
	15 mg/kg	8	0	0	8	8	21.9	0.00016	4.4	0	0	1	0	4	3	0	6	PR

<sup>1</sup> A: Control; <sup>2</sup> B: Treated; <sup>3</sup> N1 – number in group; <sup>4</sup> Nd – number that experienced toxic deaths; <sup>5</sup> No – number of additional mice excluded; <sup>6</sup> Na – number analyzed; <sup>7</sup> Number of events. An event was defined as ≥25% hCD45 cells in the peripheral blood; <sup>8</sup> Kaplan-Meier estimate of median days to event. Time to event was estimated using interpolation.

**Table S4. *In vivo* efficacy of birinapant in combination with VXL or Enbrel against Ph-like PDXs.**

<b>Xenograft</b>	<b>Treatment</b>	<b>EFS (Days)</b>	<b>T-C (Days)</b>	<b>P-Value (vs.Comb)</b>	<b>Median ORM</b>	<b>Therapeutic Enhancement</b>
PALLSD	VXL	31.8	28.2	<i>0.0004</i>	<b>CR</b>	YES
	Bpt	12.8	9.1	<i>0.0001</i>	<b>PD2</b>	
	VXL + Bpt	39	35.3	-	<b>CR</b>	
PAKHZT	VXL	41.1	29	<i>&lt;0.0001</i>	<b>CR</b>	YES
	Bpt	43.9	31.8	<i>&lt;0.0001</i>	<b>CR</b>	
	VXL + Bpt	53	40.9	-	<b>CR</b>	
PAKHZT	Enbrel	13.4	-0.1	0.07	<b>PD1</b>	NO
	Bpt	49.1	35.6	<i>0.0001</i>	<b>CR</b>	
	Enbrel + Bpt	31.8	18.4	-	<b>CR</b>	

**Table S5. List of genes differentially expressed between birinapant Responders and Non-Responders**

Gene	p-value (R vs. NR)	Fold-Change (R vs. NR)	Fold-Change (Description)
C18ORF51	0.00755	7.54171	R up vs NR
CYB5R2	0.01799	7.29482	R up vs NR
TSPAN7	0.02410	5.04566	R up vs NR
ALOX5AP	0.00512	3.04357	R up vs NR
NPY	0.03950	2.96562	R up vs NR
BMP2	0.01360	2.9536	R up vs NR
ARHGEF10	0.01327	2.85364	R up vs NR
UMODL1	0.00461	2.7054	R up vs NR
RAG1	0.04096	2.62732	R up vs NR
SH2D4B	0.01769	2.52623	R up vs NR
TNFRSF1A	0.04875	2.51189	R up vs NR
ST3GAL6	0.00555	2.43217	R up vs NR
SPTA1	0.00742	2.40766	R up vs NR
LMO2	0.02014	2.33215	R up vs NR
CAV1	0.03048	2.29861	R up vs NR
HCST	0.03033	2.25507	R up vs NR
GSN	0.04754	2.23764	R up vs NR
DLL1	0.02507	2.20604	R up vs NR
AHI1	0.00978	2.16055	R up vs NR
HIST1H2BG	0.03997	2.14739	R up vs NR
NOS2	0.00503	2.08822	R up vs NR
NRN1	0.03971	2.08814	R up vs NR
PTPRE	0.02672	2.03646	R up vs NR
SCML2	0.01500	2.03196	R up vs NR
RNU4ATAC	0.01593	2.01283	R up vs NR
NRIP1	0.03917	1.96564	R up vs NR
NOS2A	0.00320	1.96045	R up vs NR
OVCH2	0.00469	1.9533	R up vs NR
SH3TC1	0.02078	1.9496	R up vs NR
HIST1H1C	0.03976	1.94639	R up vs NR
GHR	0.03361	1.94194	R up vs NR
HIST1H2AE	0.02020	1.94031	R up vs NR
FHIT	0.03974	1.93139	R up vs NR
IL12A	0.04626	1.91505	R up vs NR
CTNBL1	0.03991	-1.94734	R down vs NR
METTL7B	0.00721	-1.95277	R down vs NR
DNAJA4	0.03031	-1.98175	R down vs NR
GPT2	0.02435	-2.02713	R down vs NR
FERMT1	0.00776	-2.03981	R down vs NR
HOMER2	0.02259	-2.05643	R down vs NR
HOXB7	0.01208	-2.06017	R down vs NR
PRICKLE1	0.02642	-2.08388	R down vs NR
CBLB	0.00110	-2.11724	R down vs NR
MAPK12	0.00032	-2.11759	R down vs NR
CHURC1	0.01243	-2.13114	R down vs NR
MAP1A	0.01716	-2.14206	R down vs NR
CISH	0.02029	-2.14267	R down vs NR
CLYBL	0.00026	-2.14604	R down vs NR
QPRT	0.00043	-2.22279	R down vs NR
WWC1	0.03578	-2.26502	R down vs NR
RNF144B	0.02509	-2.3242	R down vs NR
NYNRIN	0.04915	-2.35449	R down vs NR

MTX2	0.03189	-2.41959	R down vs NR
NUDT14	0.00106	-2.52599	R down vs NR
KIAA0367	0.00042	-2.56347	R down vs NR
C17ORF87	0.02201	-2.60313	R down vs NR
TIMP2	0.00626	-2.8475	R down vs NR
CALD1	0.02196	-2.89648	R down vs NR
BNIP3	0.04426	-3.30204	R down vs NR
ECHDC2	0.04945	-3.34277	R down vs NR
ALDH1A1	0.04345	-3.34296	R down vs NR
SLC27A2	0.03033	-3.43106	R down vs NR
LOC644936	0.00387	-3.54171	R down vs NR
PADI4	0.01000	-3.78561	R down vs NR
LAMA5	0.02016	-3.9566	R down vs NR
LAPTM4B	0.02249	-5.12922	R down vs NR
C20ORF103	0.04087	-7.38602	R down vs NR
IGJ	0.00025	-29.446	R down vs NR

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